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Advances in antiretroviral therapy and viral load monitoring

Scott M. Hammer

Objective: To highlight recent developments in the field of antiretroviral therapy and viral load monitoring.

Methods: Review of studies detailing the efficacy of the antiretroviral agents and combinations furthest along in clinical development and the application of plasma HIV RNA quantification as a disease marker.

Results: Developments in the field of antiretroviral therapy have led to substantial advances in the approach to management of HIV-infected persons. These include the end of the zidovudine (ZDV) monotherapy era; the demonstration of a survival benefit conferred by antiretroviral therapy in patients with CD4 counts of 200–500×10⁶/l; the further development of newer nucleoside analog combinations (e.g., ZDV–lamivudine, stavudine–didanosine, stavudine–lamivudine, ZDV–1592U89) and the non-nucleoside reverse transcriptase inhibitor class of compounds; and, perhaps most importantly, the advent of the protease inhibitor era. Trials of ritonavir and saquinavir have proven that clinical benefit can be conferred by protease inhibitors, and three-drug combination regimens, such as indinavir–ZDV–lamivudine, have shown the potential for degrees of viral suppression not previously seen. Newer protease inhibitors, such as nelfinavir and VX-478/GW141W94, hold promise for further advances. The concurrent development of assays to quantitatively measure plasma HIV RNA has provided laboratory tools to improve our understanding of disease pathogenesis, to assess the *in vivo* potency of treatment regimens and to characterize the risk of disease progression.

Conclusions: Recent progress in HIV disease pathogenesis, antiretroviral therapy and viral load monitoring indicates the interdependence of these factors. The current optimism in the field is warranted but complex challenges must be met if the fulfilment of this hope is to be realized by the world community.

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Keywords: Reverse transcriptase inhibitors, protease inhibitors, plasma HIV RNA

Introduction

The field of antiretroviral therapeutics has entered its second decade accompanied by a wave of unprecedented enthusiasm. This renewed sense of optimism that greater and greater control of HIV disease can be achieved is a reflection of a growing list of accomplishments which have been seen in the past 2 years. These include the following: (1) the results of four major clinical endpoint trials in adults and children which have contributed to the end of the zidovudine (ZDV) monotherapy era as a standard of care [1–4]; (2) proof of the clinical benefit of combination therapy confirming the long-held hypothesis that this would hold true [1–3,5]; (3) demonstration of a survival benefit conferred by antiretroviral therapy in individuals with intermediate-stage disease as characterized by a CD4 cell count between 200 and 500·10⁶/l [2]. Previous controlled clinical trials had only established that a survival benefit with therapy was evident in patients with advanced disease or those with a CD4 cell count under 200·10⁶/l [6]. (4) Most significantly, the advent of the protease inhibitor era which has brought with it the demonstration of the ability to suppress viral replication to levels not previously seen. (5) Continued development of

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new agents with the potential for imminent clinical impact including newer protease inhibitors and reverse transcriptase (RT) inhibitors of the nucleoside, non-nucleoside, and acyclic nucleoside phosphonate classes. (6) Exploration of newer combinations such as double protease inhibitor therapy. (7) Demonstration of a two-thirds reduction in the rate of maternal–fetal HIV transmission with ZDV and the consequent hope that even further reductions will ensue with more potent regimens [7]. This is one of the most important recent developments and one that truly has the potential for global impact. (8) Demonstration, in a retrospective case–control study, of the prophylactic benefit of antiretroviral intervention in high risk occupational exposures with a 79% hazard reduction seen [8]. (9) Finally, mounting evidence for a beneficial effect of treating primary infection. This was first reported for ZDV monotherapy and more potent combination regimens have resulted in striking virologic results carrying with it the potential for changing the early viral setpoint established after primary infection [9,10]. Such impressive virologic results have, in part, contributed to the consideration of the hypothesis that viral eradication might be possible.

Pathogenesis, therapy and viral load monitoring: an interdependent triad

These advances would not have been possible without a greater understanding of the disease process, a firmer grounding of the principles of therapy in pathogenetic concepts and the availability of viral load monitoring along with the demonstration of its predictive value for clinical outcome. Fig. 1 illustrates the interdependence of HIV pathogenesis, antiretroviral therapy and viral load monitoring. As an illustration of this notion, the availability of potent agents such as the protease inhibitors and the ability to accurately quantify plasma HIV RNA were directly responsible for the breakthroughs in viral dynamics reported by Ho *et al.* [11], Wei *et al.* [12] and Perelson *et al.* [13]. Conversely, the newer pathogenetic concepts detailing the high level of viral replication present throughout the course of disease have promoted the study and clinical use of increasingly potent regimens, and our ability to quantify the effect of these treatments has been a direct result of the availability of plasma HIV RNA testing. Thus, these three aspects of the field cannot, and should not, be separated from one another.

Combination therapy

The argument that combination therapy would ultimately be the best approach to the management of HIV disease has been put forth ever since agents beyond ZDV became available, and although supported by a number of phase II trials demonstrating greater marker responses with combination therapy [14–16], it is only recently that the clinical superiority of this approach over ZDV monotherapy has been proven. The rationale supporting the combination therapy approach has been often stated and encompasses four essential elements: (1) to provide additive or synergistic antiviral activity; (2) to modulate, and hopefully prevent, the emergence of resistance; (3) to minimize toxicity; and (4) to provide drug activity in different cellular and body compartments [17]. The recent improvement in our understanding of viral pathogenesis — most specifically, the explosion of information concerning viral dynamics [11–13,18,19] — has positioned a combination approach to treatment as the most logical given the proliferative nature of the viral replicative process and the potential for escape from single agent therapy.

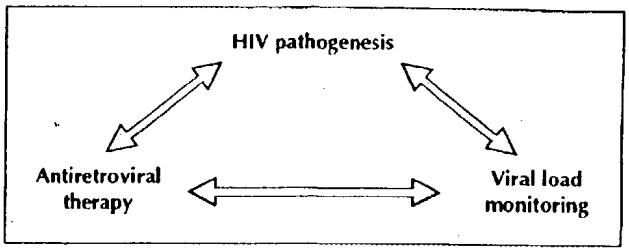

Fig. 1. Representation of the interdependence of HIV disease pathogenesis, antiretroviral therapy and viral load monitoring.

Antiretroviral therapy

Nucleoside analog reverse transcriptase inhibitors

The first class of agents developed, and still a cornerstone of therapy, are the nucleoside analog RT inhibitors (Table 1). In some countries, five nucleoside analogs are currently approved. These include ZDV, didanosine (ddI), zalcitabine (ddC), stavudine (d4T) and lamivudine (3TC) (Fig. 2). A sixth agent currently in development, 1592U89, is a carbocyclic nucleoside analog which has shown promise on the basis of phase II clinical trial data demonstrating an encouraging activity and safety profile [20]. Within the past year, four major trials in both adults and children

Table 1. Antiretroviral agents approved or furthest in clinical development.

Nucleoside analog reverse transcriptase inhibitors
Zidovudine
Didanosine
Zalcitabine
Stavudine
Lamivudine
1592U89
Non-nucleoside (HIV-1-specific) reverse transcriptase inhibitors
Nevirapine
Delavirdine
Loviride
DMP-266
Acyclic nucleoside phosphonate reverse transcriptase inhibitors
Adefovir
Protease inhibitors
Saquinavir
Ritonavir
Indinavir
Nelfinavir
VX-478/GW141W94

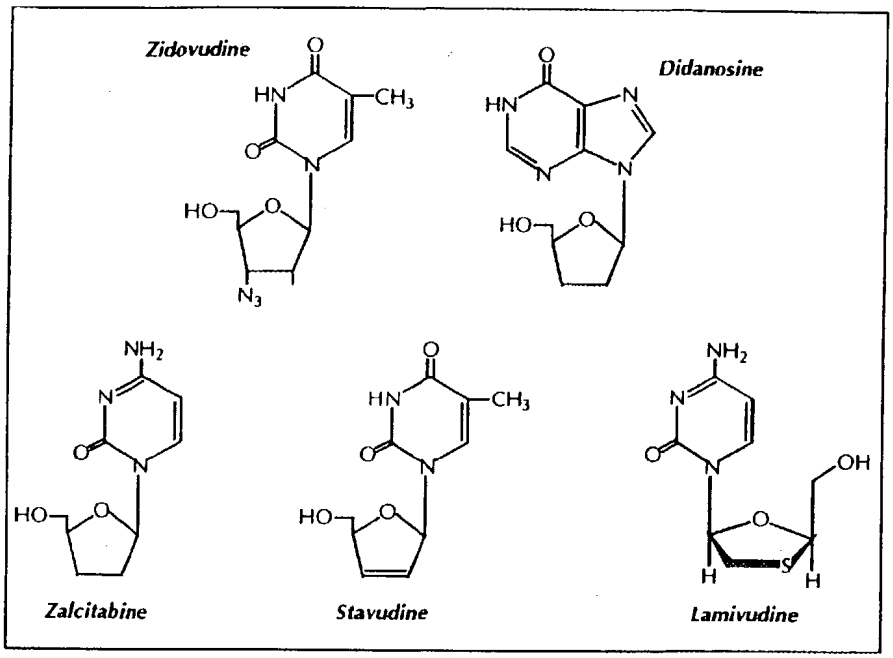

Fig. 2. Chemical structures of the nucleoside analog reverse transcriptase inhibitors.

examining the clinical efficacy of combinations of the first generation of nucleoside analogs — ZDV plus ddI or ZDV plus ddC — have been reported. AIDS Clinical Trials Group (ACTG) 175, Delta and NuCombo [1–3] were adult studies that enrolled subjects with CD4 counts of 200–500, <350 and <200×10⁶/l, respectively, both ZDV-naive and experienced, and compared ZDV–ddI or ZDV–ddC with ZDV alone; ACTG 175 also contained a ddI monotherapy arm [1–3]. ACTG 152 enrolled children aged 3 months to 18 years with little or no prior ZDV experience and compared monotherapy with ZDV or ddI with ZDV–ddI combination therapy [4]. Consistently across these four trials, the combination of ZDV–ddI was found to be superior to ZDV in ZDV-naive subjects. In ZDV-experienced subjects, ZDV–ddI was most clearly superior in ACTG 175, but a survival benefit was also demonstrated in the Delta study [1,2]. Of note, ddI monotherapy, which was studied in both ACTG 175 and ACTG 152, was comparable with respect to clinical endpoints, although the marker responses in ACTG 175 favored the combination [21]. The combination of ZDV–ddC was also found to be clearly superior to ZDV monotherapy in ZDV-naive subjects in the ACTG 175 and Delta trials, although in patients with extensive ZDV experience, the addition of ddC to prior ZDV monotherapy confers no additional clinical benefit. The lack of efficacy of ZDV–ddC in ZDV-experienced subjects seen in the Delta, NuCombo and ACTG 175 studies, confirms the previously reported results of ACTG 155, a trial which compared ZDV–ddC versus ZDV or ddC monotherapy in individuals with CD4 cell counts <300×10⁶/l [22]. The simple addition of ddC to ZDV in ZDV-experienced patients can no longer be considered a standard of care.

Table 2. Reductions in risk of disease progression: AIDS Clinical Trials Group (ACTG) 175, Delta and NuCombo studies [1–3].

	%		
	ACTG 175 (CD4 200–500)†	Delta (CD4 <350)†	NuCombo (CD4 <200)†
ZDV–ddI versus ZDV			
AIDS or death	36*	27*	14
Death	45*	33*	12
ZDV–ddC versus ZDV			
AIDS or death	23	14*	8
Death	29	21*	4

*Statistically significant. †×10⁶/l. Results shown include all patients [zidovudine (ZDV)-naive and experienced]. ddI, Didanosine; ddC, zalcitabine; AIDS, new AIDS-defining event.

The reductions in risk of disease progression for the three major adult trials of ZDV–ddI and ZDV–ddC are listed in Table 2. These three studies comprise over 6700 patients and the aggregate results, encompassing both ZDV-naive and experienced subjects, are shown in order to provide a broad view of nucleoside combination versus ZDV monotherapy. Reductions in the risk of disease progression or death are seen consistently in favor of the combination of ZDV–ddI, but when viewed across the stages of disease reflected in the three studies, one sees that the percentage reductions in disease progression diminish as one moves from the earlier (ACTG 175) to the later (Delta and NuCombo) stage populations. It should be noted again that ddI monotherapy performed comparably to ZDV–ddI in ACTG 175, but if one looks at these studies in the aggregate, the inferiority of ZDV monotherapy over the combination regimen is clear. In addition, the demonstration of a 45% reduction in the risk of death with ZDV–ddI in the intermediate disease stage population studied in ACTG 175 heralds the important message that

even drugs of modest activity can be clinically efficacious and that antiretroviral therapy should not be reserved for only symptomatic or advanced disease stage patients. When ZDV–ddC is viewed across these three trials (Table 2), one sees that the reductions in risk of disease progression are overall somewhat lower than for ZDV–ddI, although the trend that the greatest reductions in risk are seen in earlier disease stages is once again evident. It should be noted that the combination of ZDV–ddC conferred a significant reduction in the risk of AIDS or death for naive subjects in ACTG 175. ZDV–ddI and ZDV–ddC as 'first generation' combination therapies are likely to be surpassed in this rapidly moving field, although the larger message of these three clinical endpoint trials should not be dismissed. They have led to the end of the ZDV monotherapy era, proven the clinical benefit of combination therapy and re-established an aggressive approach to therapy in patients with CD4 cell counts below 500×10⁶/l.

Despite the importance of the ZDV–ddI and ZDV–ddC studies, the future is clearly with newer agents and combinations, and one of the most promising and widely prescribed combination regimens is that of ZDV plus 3TC. In four phase II studies in both ZDV-naive and experienced subjects, this combination was shown to produce greater and more sustained viral load reductions and CD4 cell increases than the control arms of ZDV alone or ZDV–ddC [23–26]. The mechanism of this efficacy is interesting and probably multifactorial. The two most important mechanisms are the synergy of the drug combination and the resistance mutational interactions [27]. The administration of 3TC, either alone or in combination with ZDV, uniformly results in the development of high-level 3TC resistance (500–1000-fold decrease in susceptibility) which is mediated by the insertion of a M184V mutation in the viral RT [28–30]. The presence of this mutation is associated with a delay in the emergence of ZDV resistance in ZDV-naive subjects and with the restoration of ZDV susceptibility in subjects already possessing ZDV-associated resistance mutations [27,31]. However, these effects are not absolute and clinical isolates which exhibit high-level, dual resistance to both ZDV and 3TC have been described [32]. Other mechanisms that may be operative to explain the *in vivo* efficacy of this combination include the increased fidelity of the RT conferred by the M184V 3TC-associated resistance mutation described by Wainberg *et al.* [33] and whether the fitness of the virus is affected by this mutation. Recently, two datasets have emerged which provide data concerning the clinical efficacy of this combination. The first involves a meta-analysis of the four phase II studies, in which 49 and 65% reductions in Centers for Disease Control and Prevention class B and class C events, respectively, were seen in the ZDV–3TC arms compared to the control arms [34]. The second dataset derives from the recently released results of the Caesar Trial (NUCB 3007) [35]. This study was a randomized, double-blind comparison of 3TC or 3TC plus loviride versus placebo added to therapy with ZDV, ZDV–ddI or ZDV–ddC in HIV-infected subjects with CD4 cell counts between 25 and 250×10⁶/l. The rate of AIDS of death was reduced from 17% in the placebo arm to 9% in the 3TC arm [hazard ratio, 0.475; 95% confidence interval (CI), 0.338–0.665; $P<0.0001$]. Mortality was also significantly reduced from 4.6 to 2.4% in the placebo and 3TC arms, respectively. In analyses of the overall study population, no additional benefit was conferred by the addition of loviride to 3TC [35]. The Caesar trial has provided strong clinical endpoint confirmation for the use of 3TC in combination with ZDV.

In addition to ZDV–3TC, other double nucleoside analog combinations are being actively studied and are gaining prominence in clinical practice. In a phase II trial, the combination of ddI and d4T has resulted in a mean 1.3 $\log_{10}$ plasma HIV RNA reduction and little neurotoxicity at 1 year in patients with CD4 counts of 200–500×10⁶/l [36]. The degree of clinical benefit seen with ddI–d4T and the risk of neurotoxicity in patients with more advanced disease are still to be defined. Similarly, combinations of d4T–3TC, ddI–3TC and d4T–ZDV are being used in clinical practice, although efficacy data from controlled trials have not yet emerged. The new nucleoside analog, 1592U89, has shown promise in a phase II study of this agent administered either as monotherapy or in combination with ZDV. Approximate 2 $\log_{10}$ plasma RNA reductions and good tolerance have been reported [20].

Non-nucleoside reverse transcriptase inhibitors

The HIV-1-specific RT inhibitors or more commonly termed non-nucleoside RT inhibitors (NNRTI) represent an intriguing class of antiretroviral agents. The lead compound in this class is nevirapine (NVP), and its introduction was marked by great enthusiasm because of the high degree of potency (50% inhibitory concentrations in the nanomolar range) and its high *in vitro* therapeutic-to-toxic ratio [37]. This early promise was quickly mitigated, however, when clinical trials of NVP monotherapy and ZDV–NVP combination therapy showed the rapid emergence of high level NVP resistance [38]. Although these early clinical trial results were disappointing, they provided some of the earliest hints of the rapidity of virus turnover and heralded future studies that were to revolutionize the world's thinking about the pathogenesis of this disease [11–13,19]. Numerous compounds of the NNRTI class have been described in preclinical testing with four having come furthest along in clinical development: nevirapine, delavirdine, loviride and DMP-266 (Table 1, Fig. 3). Illustrative of the resurgence of the potential of this drug class are the results of the Boehringer Ingelheim study 1046 [39]. In this trial of antiretroviral-naive subjects with CD4 cell counts between 200 and 600×10⁶/l, nearly 75% of individuals treated with the triple combination of NVP–ZDV–ddI have been shown to have an undetectable plasma HIV RNA level at 28 weeks [39], a

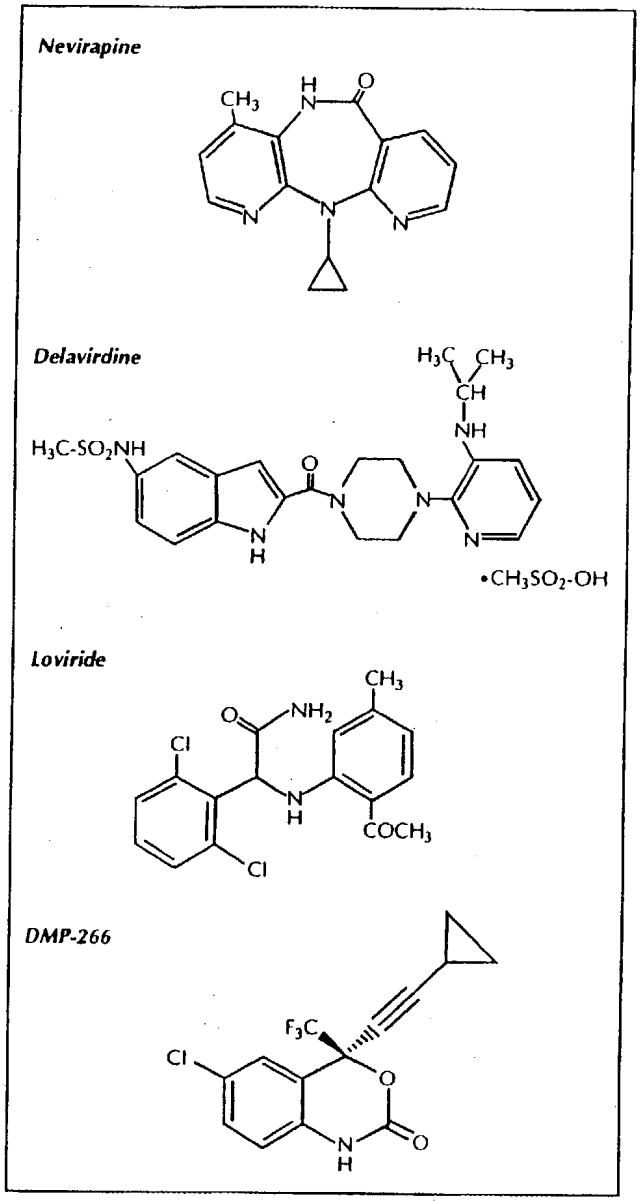

Fig. 3. Chemical structures of the non-nucleoside (HIV-1-specific) reverse transcriptase inhibitors.

proportion close to that reported for triple combination regimens containing protease inhibitors [40]. Furthermore, although the number of clinical isolates derived from patients in this trial studied is small, in drug-adherent subjects NVP susceptibility has been maintained [39]. This experience suggests that the appropriate use of this class of agents is as a component of combination regimens containing other drugs to which the patient has not been previously exposed. It also raises the strategic question of the role of this class of compounds as part of initial treatment regimens rather than exclusively as alternative treatments. Drug efficacy may also be enhanced by maintaining *in vivo* drug concentrations above the 50% inhibitory concentrations of resistant isolates [41,42]. This class of agents may also have a strategic advantage when employed as part of short-term prophylactic regimens

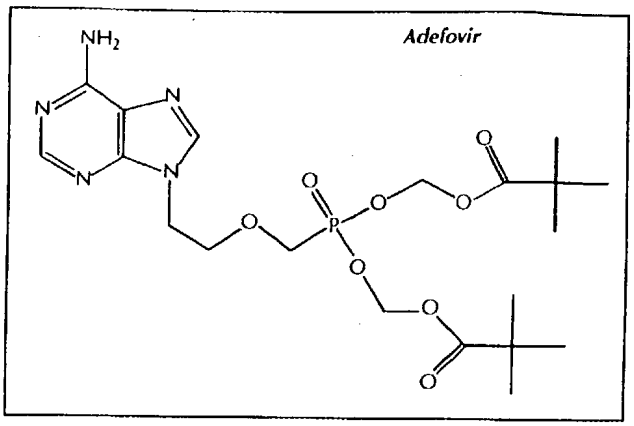

Fig. 4. Chemical structure of the acyclic nucleoside phosphonate, adefovir.

following accidental HIV exposure or the attempted interruption of maternal–fetal transmission. In these settings, the high degree of potency of the parent compound which does not need to undergo intracellular metabolism for activity is an advantage, and the potential for the rapid emergence of resistance is less of an issue.

Acyclic nucleoside phosphonate reverse transcriptase inhibitors

The acyclic nucleoside phosphonates are a class of agents that have activity against herpesviruses, hepadnaviruses and retroviruses [43]. The lead antiretroviral compound in this class is adefovir, the bis-pivaloyloxymethyl ester derivative of 9-[2-phosphonylmethoxyethyl]adenine (Table 1, Fig. 4). This agent has shown moderate marker activity (CD4 and plasma HIV RNA) in early trials [44] and holds promise as a component of combination regimens. Another related compound, (R)-9-(2-phosphonylmethoxypropyl)-adenine, has been shown to have marked prophylactic efficacy in the simian immunodeficiency virus macaque model [45].

Protease inhibitors

One can reasonably argue that 1995–1996 has been a watershed in the field of antiretroviral therapy and this, in large measure, is due to the successful clinical development of a class of compounds directed at the HIV aspartyl protease. This is a 99 amino-acid homodimer that is essential for virus maturation and infectivity [46–48]. The drug development process directed at this promising target has validated the approach of 'rational' drug design for HIV therapeutics. The protease inhibitors that have either been approved or are furthest along in clinical development are listed in Table 1 (chemical structures are shown in Fig. 5).

Saquinavir (SQV) was the first protease inhibitor approved for the treatment of HIV infection, receiving approval in the United States in late 1995. Clinical trials of SQV have shown that the combination of SQV–ZDV can raise CD4 counts more durably than either drug alone

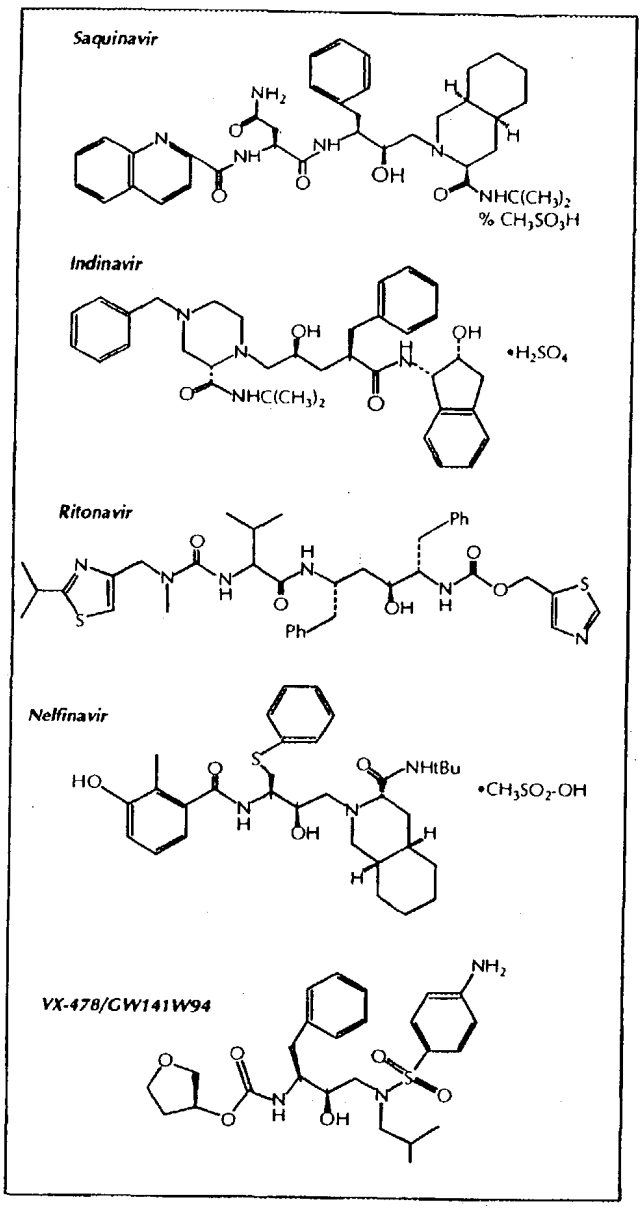

Fig. 5. Chemical structures of the protease inhibitors.

in ZDV-naive subjects [49,50] and the triple combination of SQV–ZDV–ddC can raise CD4 counts and lower quantitative measures of virus load to a greater degree than either SQV–ddC or ZDV–ddC in ZDV-experienced patients, although the marker responses in the latter study (ACTG 229) were modest [51]. In a recently reported clinical endpoint study (Hoffmann-La Roche study NV 14256), the combination of SQV–ddC was found to confer a clinical benefit over ZDV or ddC alone in ZDV-experienced subjects with CD4 counts of 50–300×10^6/l [52]. AIDS or death events were reduced by approximately 50% and the mortality was reduced by two-thirds in the SQV–ddC arm compared to ddC alone [52]. Although the comparison arms in this trial (ddC or SQV monotherapy) are of relatively modest potency by current standards, the results clearly show a clinical benefit conferred by the SQV–ddC combination arm, making this the second trial of a protease inhibitor in which a positive effect on disease progression and survival has been shown. The major concern regarding SQV has been the low oral bioavailability (4% with food) of the approved formulation which limits its *in vivo* potency [53]. This challenge is being met through the development of a new formulation with enhanced bioavailability and by taking advantage of the fact that CYP3A4 P450 isozyme inhibitors can increase SQV levels. This frames part of the rationale for employing the combination of ritonavir (RTV) and SQV since RTV is a potent P450 inhibitor. Varying doses of RTV and SQV within this double protease inhibitor combination are being tested, but preliminarily it appears that either 400 or 600 mg RTV twice daily can be combined safely and effectively with 600 mg SQV twice daily [54].

RTV and indinavir (IDV) have substantial and comparable *in vivo* potency and the results of clinical trials with these agents are at the heart of the current optimism in this field. Monotherapy with either of these agents results in impressive marker responses with median rises in CD4 cells of approximately 100×10^6/l and 2 $\log_{10}$ declines in plasma HIV RNA [55–57]. Among the numerous trials of these agents, two are of particular note: the Abbott 247 trial [58] of RTV versus placebo in advanced HIV disease and the Merck 035 trial [40] of the triple combination of ZDV–3TC–IDV. In the Abbott 247 trial, 1090 patients with CD4 cell counts <50×10^6/l were randomly assigned to RTV or placebo on a substrate of stable, 'standard of care' nucleoside therapy. In recently updated data, the progression rate to AIDS or death was reduced from 37% in the placebo group to 20% in the RTV group (hazard ratio, 0.51; 95% CI, 0.40–0.64; P<0.001) and mortality was reduced from 19 to 13% (hazard ratio, 0.64; 95% CI, 0.47–0.87; P<0.005) [58]. The major issue raised when the results of this study were first announced was whether durability of benefit would be seen, since the median follow-up time when the blinded phase of the trial was stopped was only approximately 6 months. However, the continued follow-up of this patient cohort suggests an ongoing benefit and other studies in progress or planned will address the critical question of durability.

The Merck 035 trial is a phase II trial that randomized ZDV-experienced subjects with CD4 counts of 50–400×10^6/l to one of three arms: IDV alone, ZDV–3TC and the triple combination of IDV–ZDV–3TC. In the triple combination arm, approximately 90% of subjects have had plasma RNA concentrations sustained below the level of detection (<500 copies/ml) through 48 weeks of follow-up, although the number of patients followed through 48 weeks so far is small [40]. This landmark study has created a standard upon which the marker efficacy of other

Table 3. Protease inhibitors: genotypic resistance profiles.

Drug	Key mutation	Additional mutations
Saquinavir	L90M	G48V
Ritonavir	V82A/F/T	I54V, A71V, M36I, K20N/R, M46I, I84V, L33F, L90M
Indinavir	V82A/F/T	M46I/L, L10I/V/R, K20M/R/I/L, L24I, I54V, L63P, I64V, A71V/T, I84V, L90M
Nelfinavir	D30N	N88D, V77I, M46I, M36I, A71T/V
VX-478/GW141W94	I50V*	I47V, M46I/L*

**In vitro* passage.

regimens is being compared. That viral suppression is complete or near complete in this regimen is illustrated by the fact that resistance to 3TC can be prevented, whereas it uniformly emerges when subjects are treated with the dual nucleoside regimen of ZDV–3TC [27,31,32]. Potent suppression of viral suppression has also been seen with combination regimens employing RTV, SQV and nelfinavir (NFV) [10,59,60].

The Achilles heel of any anti-infective agent is the development of resistance, and this is as true for protease inhibitors as it is for other classes of antiretroviral agents. The predominant mechanism by which resistance to protease inhibitors develops is through the emergence of mutations at the active site of the enzyme, which results in diminished binding of the inhibitor, but other potential mechanisms include mutations which increase the enzymatic efficiency of the protease molecule and mutational changes which affect the target cleavage sites of the protease enzyme. In studies of RTV and IDV, at least three to four mutations need to accumulate before phenotypic resistance can be demonstrated *in vitro* [61–64]. Table 3 lists the key as well as the secondary mutations that have been described to mediate genotypic resistance to the protease inhibitors furthest along in clinical development. The key mutation for SQV is L90M, which occurs in approximately 45% of patients treated with monotherapy for 1 year [65]. RTV and IDV share a key resistance mutation —V82A/F/T — and there is overlap with many of the additional mutations, explaining the near complete cross-resistance seen with these agents. For RTV, the mutation at V82 typically is the first mutation identified, although this appears not to be the case for IDV [62,63]. Although it is typically present in clinical isolates with phenotypic IDV resistance, it is not necessarily the first mutation identified [63]. Interestingly, a unique mutation has been described for NFV, D30N, which arises both with *in vitro* passage of HIV-1 in the presence of NFV and in isolates derived from treated patients. The D30N mutation does not appear to confer cross-resistance to other protease inhibitors [66]. VX-478/GW141W94 also may induce a more unique resistance profile with the key mutation being I50V, although the data supporting this are derived from *in vitro* studies and information from isolates derived from treated patients is awaited [67]. Adding to the complexity of the cross-protease inhibitor resistance debate is the fact that the order in which these agents is administered may determine whether cross-resistance occurs. For example, patients treated with RTV whose isolates develop multiple RTV-associated resistance mutations may exhibit cross-resistance to NFV, but initial treatment with NFV may yield NFV-resistant isolates that remain susceptible to RTV [62,66]. This once again illustrates that the choice of an agent within a drug class may have important implications for the subsequent therapeutic options. This is a complex and critically important field of research and it is only through well-designed clinical trials that the proper sequencing and combinations of these agents will be delineated.

The protease inhibitor era, although still in its infancy, has taught us a number of important lessons. These include (1) potent suppression of virus replication to levels below detection in plasma can be achieved; (2) impressive degrees of CD4 cell increases, of the order of $100–200\times10^6$/l, which are durable and progressive can be seen; (3) prevention of resistance is achievable by potent virus suppression; and (4) eradication of virus has become an acceptable hypothesis to test — a concept that 1 year ago would have been deemed by many much too far reaching, if not dismissed entirely.

Viral load monitoring

As mentioned above, the ability to precisely quantify plasma HIV RNA has provided the tool to facilitate progress in understanding disease pathogenesis and developing improved therapies. Methods which rely on either target amplification, such as the RT-polymerase chain reaction (RT-PCR)-based systems, or signal amplification, illustrated by the branched chain DNA (bDNA) technology (Fig. 6), are becoming more and more widely available to both clinicians and researchers [68]. Plasma HIV RNA is detectable at all stages of disease in most untreated patients with the most sensitive assays available [69]. It largely reflects replication in the lymphoreticular tissues more so than it does the production of virus in peripheral blood mononuclear cells. In addition to the new insights provided into viral dynamics, plasma HIV RNA quantification has confirmed what other virologic measures had previously suggested — that the CD4 cell count has a low correlation with virus load. A number of studies, including the Multicenter AIDS Cohort Study (MACS) and the virology substudy of ACTG 175, have shown a significant but low degree of correlation between the CD4 cell count and the plasma HIV RNA concentration. Although there are generally higher plasma HIV RNA concentrations seen at lower CD4 cell counts, at any particular CD4 count category the plasma HIV RNA in a population may vary by 2–3 logs [21,70]. These studies have made it increasingly clear that the CD4 cell count is at best a gross measure of where a patient stands virologically in the course of HIV disease.

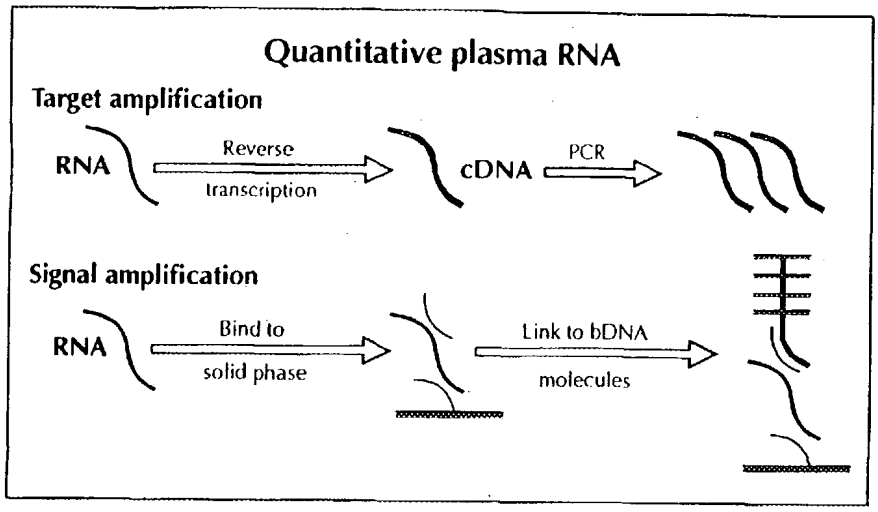

Fig. 6. Schematic representation of target and signal amplification methodologies for the quantification of plasma HIV RNA. PCR, Polymerase chain reaction; bDNA, branched chain DNA.

As with any new or evolving laboratory parameter in the HIV disease field, immediate attention has been focused on trying to define the utility of plasma HIV RNA quantification as a disease marker. There are three components to its marker applications to consider: its utility as a natural history (prognostic) marker, its ability to reflect drug activity in the short term, and its degree of surrogacy. With respect to prognosis, levels of plasma HIV RNA at the time of seroconversion and baseline levels in well-established disease both correlate with the risk of disease progression [70–72]. Among the most influential prognostic data to emerge in the past year have been those derived from the MACS. In the Pittsburgh cohort of the MACS, Mellors *et al.* [70] analyzed disease progression and survival in relation to baseline plasma HIV concentrations for 180 subjects. When divided into quartiles, a >70% survival at 10 years was noted for the quartile of subjects who had ≤4530 copies/ml of HIV RNA at baseline versus a <25% 10-year survival for those with a baseline concentration of >36 270 copies/ml [70]. This dataset also demonstrated the differential predictive power of HIV RNA over CD4 in patients with earlier stage disease. For example, in subjects with CD4 cell counts $>500\times10^6$/l at baseline, individuals with baseline plasma HIV RNA concentrations ≤10 190 copies/ml and >10 190 copies/ml had 10-year survivals of approximately 70 and 25%, respectively [70]. These data have been extended by Mellors *et al.* [73] in a MACS-wide study of 1604 patients. An 18.5-fold increase in the relative hazard of death was noted for the patient category exhibiting a baseline plasma HIV RNA concentration of >30 000 copies/ml compared to the group with RNA copy numbers <500 copies/ml [73]. Furthermore, differences between groups with relatively low copy numbers have been teased out by these data with patients in the 500–3000 RNA copy number category exhibiting a 2.8-fold higher risk of death than those in the <500 copies/ml category [73].

As noted, a second component of a useful HIV disease marker is as a mirror of *in vivo* biologic drug activity. In this regard, plasma HIV RNA is clearly a superb reflection of drug activity in the short term when an effective antiretroviral regimen is introduced [11,12]. The third and most elusive component of any marker is its degree of surrogacy: the percentage of a particular treatment effect that is explained by a change in the marker. A number of datasets have emerged from controlled clinical trials to indicate that early declines of plasma HIV RNA, irrespective of a particular treatment arm, correlate with a diminished risk of disease progression [21,74–76]. In the ACTG 175 virology substudy, for example, each 1 $\log_{10}$ decrease in plasma HIV RNA at 8 weeks into the study was associated with a 65% decrease in the risk of disease progression and a 71% decrease in the risk of death, and, in this population, was more powerful as a predictor of outcome than the CD4 cell count in multivariate modelling [21]. For specific treatments, the data are much more limited. In the case of ZDV, O'Brien *et al.* [77] have reported that a decrease of 75% in the plasma HIV RNA concentration in the first 6 months of treatment explains 59% of the treatment effect. Much more data are needed in this regard for the other nucleoside analogs, protease inhibitors and combination regimens. Such data will have major implications for clinical practice and potentially for the drug approval process.

In addition to the prognostic information provided by plasma HIV RNA quantification, it also has the potential for immediate diagnostic and therapeutic applications. It should prove useful as an adjunct in neonatal diagnosis, in closing the asymptomatic window period following HIV exposure and in diagnosing an acute symptomatic retroviral syndrome prior to seroconversion. In therapeutic decision-making, plasma HIV RNA quantification can assist with the decision to initiate therapy in patients with higher CD4 cell counts (e.g., $>550\times10^6$/l), as the MACS data suggest [70]. In patients starting a new antiretroviral regimen, a value 2–4 weeks after initiation would indicate whether a biologic response has been achieved and its virologic impact. In patients appearing stable on longer term treat-

ment by clinical and CD4 cell parameters, it might assist with the decision to change therapy if a level associated with higher risk were obtained and further therapeutic options existed. It may also have a role in monitoring pregnant women to try to decrease the risk of maternal–fetal HIV transmission. However, it should be noted that the maternal viral load is only one of a number of factors involved in determining this risk and there is no lower limit threshold of maternal plasma HIV RNA level below which transmission does not occur [60]. Additional general caveats concerning the application of viral load monitoring in clinical practice are worth mentioning. These include assay performance issues such as specimen handling, the reliability of the performing laboratory, for example, and biologic perturbations such as intercurrent illnesses and vaccinations that can temporarily alter the HIV RNA level [68,78]. It also needs to be interpreted, of course, in the overall clinical context of the patient.

Concluding perspectives

In order to continue the progress in antiretroviral therapy witnessed over the past 2 years, a number of aims need to be realized. These include (1) defining of appropriate therapeutic strategies including the use of immediate versus deferred protease inhibitor containing regimens in patients with earlier stage disease, the proper sequencing of protease inhibitors to maximize their benefit and minimize cross resistance, and the place of the NNRTIs as part of initial or alternative regimens; (2) defining newer combinations, including double protease inhibitor treatment and protease inhibitors combined with NNRTIs in two, three and possibly four-drug regimens; (3) continued development of new classes of agents such as HIV integrase or zinc-finger inhibitors [79,80], or agents that exploit new basic discoveries in virus–cell interactions, such as modalities which would interrupt HIV–fusin (leukocyte-derived seven-transmembrane domain receptor) or HIV–cysteine-cysteine chemokine receptor CKR-5 interactions [81–84]. Efforts to inhibit Tat and Rev function through chemotherapeutic agents or gene therapy approaches need to continue. (4) Finally, careful testing of the hypothesis that HIV eradication can be achieved in both the setting of primary and established infection.

General principles regarding the approach to antiretroviral therapy can be summarized as follows:

(1) Choice of regimen should be based on the following: baseline clinical, immunologic (CD4) and virologic (HIV RNA) status of patient; potency of regimen and supporting clinical trial data; side-effect and drug interaction profile; prior therapy, if any; predicted adherence to regimen; potential impact on future treatment options; and cost.

(2) Two and three-drug combinations regimens will increasingly reflect the standard of care: nucleoside analog combinations still have an important role; and protease inhibitor and NNRTI-containing regimens will continue to gain in prominence and will have roles as both initial and alternative treatments.

(3) When changing therapy because of treatment failure, attempt to introduce at least two new agents.

(4) Strategies of treatment, not individual regimens, are the key to long-term management.

As with all developments in the field of HIV disease, the current wave of optimism needs to be kept in perspective and, although optimism is warranted, it needs to be tempered with caution. Research needs to establish whether the new potent regimens are achieving the same level of virus suppression in lymphoreticular tissues, the central nervous system and the genital tract that are being seen in the peripheral blood compartment. The degree of meaningful clinical benefit of the newer regimens and strategies needs to be defined. Most importantly, complacency needs to be avoided, and, more appropriately, the recent advances should spur even more intensive drug development efforts and the testing of more aggressive therapeutic approaches.

As one contemplates the 'One World, One Hope' theme of the *XI International Conference on AIDS* in Vancouver, it is well to remember that recent developments have resulted from a multinational research effort which has created a unifying spirit of optimism and hope for the future. However, access and cost are, and will remain, major obstacles to underserved populations throughout the world. If the promise of recent developments is realized, a moral imperative to make cost-effective therapies widely available will face governments, non-governmental organizations and pharmaceutical manufacturers throughout the world until preventive efforts such as vaccine development reach their maturity.

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